

DRAFT: August 31, 1994

DECISION DOCUMENT
TSCA SECTION 5(H)(4) EXEMPTION FOR
SACCHAROMYCES UVARUM

I. SUMMARY

Saccharomyces uvarum is one species of a genus which has an extensive history of use in the fermentation and food processing industry. S. uvarum, also called S. bayanus, is referred to as a "wine yeast" due to their broad use in the production of wines. S. uvarum has a long history of safe use in production of alcoholic beverages and industrial ethanol. Although it is expected to survive in the environment, it is not expected to cause any adverse environmental effects. Despite S. uvarum's broad use and exposure there is no reported incidence of adverse effects to either humans or the environment. While S. uvarum has been used industrially for years, specific strains have not been distinguished.

II. BACKGROUND

A. Introduction

EPA recognizes that some microorganisms present a low risk when used under specific conditions at general commercial use. Therefore, EPA is proposing expedited regulatory processes for certain microorganisms under these specific conditions at the general commercial use stage. Microorganism uses that would be exempt meet criteria addressing: (1) performance based standards for minimizing the numbers of microorganisms emitted from the manufacturing facility; (2) the introduced genetic material; and (3) the recipient microorganism. Microorganisms that qualify for these exemptions, termed Tier I and Tier II, must meet a standard of no unreasonable risk in the exempted use.

To evaluate the potential for unreasonable risk to human health or the environment in developing these exemptions, EPA focuses primarily on the characteristics of the recipient microorganisms. If the recipient is shown to have little or no potential for adverse effects, introduced genetic material meeting the specified criteria would not likely significantly increase potential for adverse effects. As further assurance that risks would be low, EPA is also specifying procedures for minimizing numbers of organisms emitted from the facility. When balanced against resource savings for society and expected product benefits, these exemptions will not present unreasonable

risks.

B. Criteria for Minimizing Release from Manufacturing Facilities

The standards prescribed for the Tier I exemption require the following: (1) the structure(s) be designed and operated to contain the microorganism, (2) access to the structure should be limited to essential personnel, (3) inactivation procedures shown to be effective in reducing the number of viable microorganisms in liquid and solid wastes should be followed prior to disposal of the wastes, (4) features to reduce microbial concentrations in aerosols and exhaust gases released from the structure should be in place, and (5) general worker hygiene and protection practices should be followed.

1. Definition of structure. EPA considers the term "structure" to refer to the building or vessel which effectively surrounds and encloses the microorganism. Vessels may have a variety of forms, e.g., cubic, ovoid, cylindrical, or spherical, and may be the fermentation vessel proper or part of the downstream product separation and purification line. All would perform the function of enclosing the microorganism. In general, the material used in the construction of such structure(s) would be impermeable, resistant to corrosion and easy to clean/sterilize. Seams, joints, fittings, associated process piping, fasteners and other similar elements would be sealed.

2. Standards to minimize microbial release. EPA is proposing, for several reasons, a somewhat cautious approach in prescribing standards for minimizing the number of microorganisms emitted through the disposal of waste and the venting of gases. First, a wide range of behaviors can be displayed by microorganisms modified consistent with EPA's standards for the introduced genetic material. Second, EPA will not conduct any review whatsoever for Tier I exemptions. EPA believes the requirement to minimize emissions will provide a measure of risk reduction necessary for making a finding of no unreasonable risk. Taken together, EPA's standards ensure that the number of microorganisms emitted from the structure is minimized.

EPA's proposed standards for minimizing emission specify that liquid and solid waste containing the microorganisms be treated to give a validated decrease in viable microbial populations so that at least 99.9999 percent of the organisms resulting from the fermentation will be killed. Since the bacteria used in fermentation processes are usually debilitated, either intentionally or through acclimation to industrial fermentation, the small fraction of microorganisms remaining

viable after inactivation treatments will likely have a reduced ability to survive during disposal or in the environment. Moreover, industrial companies, in an attempt to keep their proprietary microorganisms from competitors and to reduce the microbial numbers to those permitted by local sanitation authorities, modify the microorganisms to increase the ability of their microorganisms to survive and perform their assigned tasks in the fermentor but decrease their ability to survive in the environment external to the fermentor.

EPA requirements also address microorganisms in the exhaust from the fermentor and along the production line. To address exhaust from fermentors, EPA is proposing that the number of microorganisms in fermentor gases be reduced by at least two logs prior to the gases being exhausted from the fermentor. EPA selected this number based on an estimate of the numbers of microorganisms likely to be in the exhaust from an uncontrolled fermentor and common industry practice. Moreover, microorganisms that are physiologically acclimated to the growth conditions within the fermentor are likely to be compromised in their ability to survive aerosolization. EPA anticipates, therefore, that few microorganisms will survive the stresses of aerosolization associated with being exhausted in a gas from the fermentor. The provision requiring reduction of microorganisms in fermentor exhaust gases contributes to minimizing the number of viable microorganisms emitted from the facility.

EPA is also proposing that the requirements specify that other systems be in place to control dissemination of microorganisms by other routes. This would include programs to control pests such as insects or rats, since these might serve as vectors for carrying microorganisms out of the fermentation facilities.

3. Worker protection. The requirement to minimize microbial emissions, in conjunction with the requirement for general worker safety and hygiene procedures, also affords a measure of protection for workers. Potential effects on workers that exist with microorganisms in general (e.g., allergenicity) will be present with the microorganisms qualifying for this exemption. As with other substances that humans may react to (e.g., pollen, chemicals, dust), the type and degree of allergenic response is determined by the biology of the exposed individual. It is unlikely that a microorganism modified in keeping with EPA's specifications for the introduced genetic material would induce a heightened response. The general worker hygiene procedures specified by EPA should protect most individuals from the allergenic responses associated with microorganisms exhausted from fermentors and/or other substances

emitted along the production line. The EPA requirement that entry be limited to essential personnel also addresses this consideration by reducing to a minimum the number of individuals exposed.

4. Effect of containment criteria. As further assurance that risks would be low, EPA is specifying procedures for minimizing numbers of organisms emitted from the facility for the Tier I exemption. EPA is not specifying standards for minimizing the number of microorganisms emitted from the facility for microorganisms qualifying for Tier II exemption. Rather, the Agency requests that submitters utilize as guidance the standards set forth for Tier I procedures. The procedures proposed by the submitter in a Tier II exemption request will be reviewed by the Agency. EPA will have the opportunity to evaluate whether the procedures the submitter intends to implement for reducing the number of organisms emitted from the facility are appropriate for that microorganism.

C. Introduced Genetic Material Criteria

In order to qualify for either Tier I or Tier II exemption, any introduced genetic material must be limited in size, well characterized, free of certain nucleotide sequences, and poorly mobilizable.

1. Limited in size. Introduced genetic material must be limited in size to consist only of the following: (1) the structural gene(s) of interest; (2) the regulatory sequences permitting the expression of solely the gene(s) of interest; (3) the associated nucleotide sequences needed to move genetic material, including linkers, homopolymers, adaptors, transposons, insertion sequences, and restriction enzyme sites; (4) the nucleotide sequences needed for vector transfer; and (5) the nucleotide sequences needed for vector maintenance.

The limited in size criterion reduces risk by excluding the introduction into a recipient of extraneous and potentially uncharacterized genetic material. The requirement that the regulatory sequences permit the expression solely of the structural gene(s) of interest reduces risk by preventing expression of genes downstream of the inserted genetic material. The limitation on the vector sequences that are components of the introduced genetic material prevents the introduction of novel traits beyond those associated with the gene(s) of interest. The overall result of the limited in size criterion is improved ability to predict the behavior of the resulting microorganism.

2. Well characterized. For introduced genetic material, well characterized means that the following have been determined: (1) the function of all of the products expressed from the structural gene(s); (2) the function of sequences that participate in the regulation of expression of the structural gene(s); and (3) the presence or absence of associated nucleotide sequences.

Well characterized includes knowledge of the function of the introduced sequences and the phenotypic expression associated with the introduced genetic material. Genetic material which has been examined at the restriction map or sequence level, but for which a function or phenotypic trait has not yet been ascribed, is not considered well characterized. Well characterized would include knowing whether multiple reading frames exist within the operon. This relates to whether more than one biological product might be encoded by a single sequence, and addresses the possibility that a modified microorganism could display unpredicted behavior should such multiple reading frames exist and their action not be anticipated.

3. Free of certain sequences. In addition to improving the ability to predict the behavior of the modified microorganism, the well characterized requirement ensures that segments encoding for either part or the whole of the toxins listed in the proposed regulatory text for the TSCA biotechnology rule would not inadvertently be introduced into the recipient microorganism.

These toxins are polypeptides of relatively high potency. Other types of toxins (e.g., modified amino acids, heterocyclic compounds, complex polysaccharides, glycoproteins, and peptides) are not listed for two reasons. First, their toxicity falls within the range of moderate to low. Second, these types of toxins generally arise from the activity of a number of genes in several metabolic pathways (multigenic).

In order for a microorganism to produce toxins of multigenic origin, a large number of different sequences would have to be introduced and appropriately expressed. It is unlikely that all of the genetic material necessary for metabolizing multigenic toxins would be inadvertently introduced into a recipient microorganism when requirements that the genetic material be limited in size and well characterized are followed.

Similarly, other properties that might present risk concerns result from the interactive expression of a large number of genes. For example, pathogenic behavior is the result of a large number of genes being appropriately expressed. Because of the

complex nature of behaviors such as pathogenicity, the probability is low that an insert consisting of well characterized, limited in size genetic material could transform the microorganisms proposed for exemption into microorganisms which display pathogenic behavior.

4. Poorly mobilizable. Poorly mobilizable means the ability of the introduced genetic material to be transferred and mobilized is inactivated, with a resulting frequency of transfer of less than 10^{-8} transfer events per recipient. The requirement that the introduced genetic material be poorly mobilizable reduces potential for transfer of introduced genetic sequences to other microorganisms in the environment. Such transfers would occur through the interaction of the introduced microorganism with indigenous microorganisms through conjugation, transduction, or transformation. Through such transfers, the introduced genetic material could be transferred to and propagated within different populations of microorganisms, including microorganisms which may never previously have been exposed to this genetic material. It is not possible to predict how the behavior of these potential recipient microorganisms will be affected after uptake and expression of the genetic material.

Since EPA is not limiting the type of organism that can serve as the source for the introduced genetic material, some limitation is placed on the ability of the introduced genetic material to be transferred. This limitation mitigates risk by significantly reducing the probability that the introduced genetic material would be transferred to and expressed by other microorganisms.

The 10^{-8} frequency is attainable given current techniques. Plasmids with transfer rates of 10^{-8} exist or are easily constructed. Some of the plasmids most commonly employed as vectors in genetic engineering (e.g., pBR325, pBR322) have mobilization/transfer frequencies of 10^{-8} or less.

The criteria set for "poorly mobilizable" for transduction and transformation should not prevent most microorganisms from meeting the exemption criteria, since the majority of transfer frequencies reported for transduction and natural transformation are less than 10^{-8} . Higher frequencies are likely only if the introduced genetic material has been altered or selected to enhance frequency.

Fungal gene transfer has also been considered in development of the poorly mobilizable criterion. Although mobile genetic elements such as transposons, plasmids and double stranded RNA exist in fungi and can be readily transferred, this transfer

usually is only possible between members of the same species during anastomosis, a process specific to fungi. Since anastomosis only occurs between members of the same species, the introduced genetic material would not be transferred to distantly related fungi as may occur with bacteria.

5. Effect of introduced genetic material criteria. The requirements placed on the introduced genetic material, in concert with the level of safety associated with Saccharomyces uvarum, ensure that the resulting microorganisms present low or negligible risk. The probability is low that the insertion of genetic material meeting EPA's criteria into strains of S. uvarum will change their behavior so that they would acquire the potential for causing adverse effects. Risks would be mitigated by the four criteria placed on the introduced genetic material, the relative safety of S. uvarum, and the inactivation criteria specified for the Tier I exemption. In the case of Tier II exemption, risks would be mitigated in light of the four criteria placed on introduced genetic material, the relative safety of S. uvarum, and EPA's review of the conditions selected.

D. Recipient Microorganism Criteria

Six criteria were used by EPA to determine eligibility of recipient microorganisms for the tiered exemption. Microorganisms which EPA finds meet these criteria are listed as eligible recipients. The first criteria would require that it be possible to clearly identify and classify the microorganism. Available genotypic and phenotypic information should allow the microorganism to be assigned without confusion to an existing taxon which is easily recognized. Second, information should be available to evaluate the relationship of the microorganism to any other closely related microorganisms which have a potential for adverse effects on human health or the environment. Third, there should be a history of commercial use for the microorganism. Fourth, the commercial uses should indicate that the microorganism products might be subject to TSCA jurisdiction. Fifth, studies are available which indicate the potential for the microorganism to cause adverse effects on human health and the environment. Sixth, studies are available which indicate the survival characteristics of the microorganism in the environment.

After each microorganism was reviewed using the six evaluation criteria, a decision was made as to whether to place the microorganism on the list. The Agency's specific determination for Saccharomyces uvarum is discussed in the next unit.

EVALUATION OF SACCHAROMYCES UVARUM

A. History of Use

1. History of safe commercial use. Saccharomyces uvarum has a history of safe use. It is widely used in the making of beer and wine and in alcohol production and is found most frequently in grape must and wine. The commercial use of the genus Saccharomyces arose from the fermentation of small grains and fruit for the production of alcoholic beverages. Schwann coined the term "Zuckerpilz" or "sugar fungus" to describe the small bodies in beer. The genus name, Saccharomyces, was derived from this term. At the present time Saccharomyces is not used for the production of so-called specialty chemicals (e.g., antibiotics, culturable enzymes, etc.); however, these yeasts are used to produce alcohol for beverages and industrial purposes.

2. Products subject to TSCA jurisdiction. EPA has not yet received a submission for use of S. uvarum under TSCA. Alcoholic beverages are regulated under statutes other than TSCA, although the use of alcohol for industrial purposes may fall under TSCA jurisdiction. There are also a limited number of reports in the literature about the production of specialty proteins. Saccharomyces is the organism of choice for the production of alcohols due to its high level of metabolic activity and its tolerance of high alcohol concentrations. The selection of species is based upon the stock (medium) used in the fermentation system and the requirement for alcoholic tolerance. With the rising costs of conventional energy sources there is a shifting to "alternative" fuels which include the generation of alcohol from various sources.

B. Identification of the Microorganism

1. Classification. Saccharomyces uvarum is, under most conditions, a poorly sporogenous yeast and falls into a category of yeasts referred to as the "wine yeasts" due to the broad utility of these fungi in the production of wines. In addition to S. uvarum, the other fungi that comprise the wine fungi are Saccharomyces cerevisiae, the yeast used in the production of beer, Saccharomyces chevalieri, Saccharomyces bayanus, and Saccharomyces italicus. The wine yeasts are characterized by both an ability to ferment sugars at a high rate and a high tolerance to alcohol. S. uvarum is a well-characterized species based on morphological and biochemical characteristics.

DNA homology studies have been employed as tools to delineate species within the genus but with conflicting results. A high degree of relatedness was found between S. uvarum and S.

bayanus. However, the DNA homology criteria was at odds with the conventional systematic criteria. Despite the high degree of homology between S. uvarum and S. bayanus, the fermentation potential of the two strains varies substantially. A reclassification of S. uvarum into S. bayanus has been reposed. While the reclassification would normally involve six previously separate species, the risk assessment addressed the component of S. bayanus that was previously classified as S. uvarum.

2. Related taxa of concern. None of these strains or other closely related species has been associated with pathogenicity toward humans or has been shown to have adverse effects on the environment.

C. Risk Summary

1. Studies regarding potential for adverse effects. S. uvarum is an organism which has an extensive history of safe use. Despite considerable use of the organism in research and the presence of S. uvarum in food, there are few reports in the literature of pathogenicity to humans or animals, and only in those cases where the human had a debilitating condition. Tests for the presence of factors associated with the virulence of yeasts (i.e., phospholipases) indicate that this organism is nonpathogenic. The organism has not been shown to produce toxins to humans. The only adverse effect noted in the literature is the presence of the "killer toxins" which are active against other strains of Saccharomyces.

2. Studies regarding survival in the environment. S. uvarum is ubiquitous in nature. It has been recovered from a variety of sites under varying ecological conditions. The organism is used in a variety of industrial scenarios. S. uvarum is commonly recovered from a variety of fresh fruits and vegetables, generally those fruits with high levels of fermentable sugars. However, it is not listed as the causative agent of food spoilage for fruits and vegetables.

IV. BENEFITS SUMMARY

Substantial benefits are associated with this proposed exemption. Saccharomyces uvarum is already widely employed in general commercial uses, some of which are subject to TSCA reporting. The Agency believes this exemption will result in resource savings both to EPA and industry without compromising the level of risk management afforded by the full 90 day review. In addition to assessing the risk of S. uvarum, EPA has developed

criteria limiting the potential for transfer of and expression of toxin sequences, and the conditions of use specified in the exemption are met (Tier I) or will be reviewed by EPA to ensure adequate risk reduction (Tier II). EPA requirements for minimizing numbers of viable microorganisms emitted are within standard operating procedures for the industry, and both the procedures and the structures specified in the exemption are the type industry uses to protect their products from contamination.

The exemption will result in reduced reporting costs and a decrease in delay associated with reporting requirements. The savings in Agency resources can be directed to reviewing activities and microorganisms which present greater uncertainty. This exemption should also facilitate development and manufacturing of new products and the accumulation of useful information.

V. RECOMMENDATION AND RATIONALE

A. Recommendation

Saccharomyces uvarum is recommended for a TSCA section 5(h)(4) tiered exemption.

B. Rationale

1. Risks from use of the recipient microorganism *S. uvarum* are low. There is an extensive history of use of and exposure to *S. uvarum* with a very limited record of adverse effects to the environment or human health. The current taxonomy of *Saccharomyces* is under revision based on the development of alternative criteria. Data suggests that only with the ingestion of high levels of *S. uvarum* or with the use of immunosuppressives can *S. uvarum* colonize in the body. Even under those conditions, there were no noted adverse effects. Releases of this microorganism to the environment through fermentation uses would not pose any significant ecological hazards, because this microorganism is ubiquitous in the environment and it is not pathogenic to animals or plants.

2. Use of strains of *S. uvarum* which are eligible for the TSCA section 5(h)(4) exemption present no unreasonable risk. The current taxonomy of *Saccharomyces* is under revision based on the development of alternative criteria. However, this should not have a major effect on the risk associated with closely related species. *Saccharomyces*, as a genus, present low risk to human health or the environment. As part of their eligibility for this

TSCA section 5(h)(4) exemption, companies are required to certify that they are using S. uvarum. It is therefore expected that companies will have information in their files which documents the correct identification of their strains.

Because the recipient microorganism was found to have little potential for adverse effects, introduced genetic material meeting the specified criteria would not likely significantly increase potential for adverse effects. As further assurance that risks would be low, EPA is specifying procedures for minimizing numbers of organisms emitted from the facility for the Tier I exemption and will be reviewing the conditions selected for the Tier II exemption. When balanced against resource savings for society and expected product benefits, this exemption will not present unreasonable risks.

Attachment 1:

INTEGRATED RISK ASSESSMENT FOR

SACCHAROMYCES UVARUM

I. INTRODUCTION

Saccharomyces uvarum is one species of a genus which has an extensive history of use in the fermentation and food processing industry. S. uvarum, also called S. bayanus, is referred to as a "wine yeast" due to their broad use in the production of wines. Taxonomically, S. uvarum is closely related to Saccharomyces cerevisiae. S. cerevisiae is used extensively in the baking of bread and the production of beer. S. cerevisiae is the subject of extensive genetic research and genomic mapping. It is expected that research on S. cerevisiae will lead to greater understanding and better characterization of the entire genus including S. uvarum. Despite S. uvarum's broad use and exposure there is no reported incidence of adverse effects to either humans or the environment.

**History of Commercial Use and Products Subject to TSCA
 Jurisdiction**

Saccharomyces uvarum has a history of safe use. It is widely used in the making of beer and wine and in alcohol production and is found most frequently in grape must and wine. The commercial use of the genus Saccharomyces arose from the fermentation of small grains and fruit for the production of

alcoholic beverages. Schwann coined the term "Zuckerpilz" or "sugar fungus" to describe the small bodies in beer. The genus name, Saccharomyces, was derived from this term. At the present time Saccharomyces is not used for the production of so-called specialty chemicals (e.g., antibiotics, culturable enzymes, etc.); however, these yeasts are used to produce alcohol for beverages and industrial purposes. Alcoholic beverages are regulated under statutes other than TSCA, although the use of alcohol for industrial purposes may fall under TSCA jurisdiction (Stewart and Russell, 1985). There are also a limited number of reports in the literature about the production of specialty proteins (El-Refai et al., 1985).

Saccharomyces is the organism of choice for the production of alcohols due to its high level of metabolic activity and its tolerance of high alcohol concentrations. The selection of species is based upon the stock (medium) used in the fermentation system and the requirement for alcoholic tolerance. With the rising costs of conventional energy sources there is a shifting to "alternative" fuels which include the generation of alcohol from various sources (Stewart and Russell, 1985).

II. IDENTIFICATION AND CLASSIFICATION

A. Taxonomy and Characterization

Saccharomyces uvarum is, under most conditions, a poorly sporogenous yeast most commonly used in the production of alcoholic beverages and vitamin assays. This organism falls into the category of yeasts referred to as the "wine yeasts" due to the broad utility of these fungi in the production of wines. In addition to S. uvarum, the other fungi that comprise the wine fungi are Saccharomyces cerevisiae, the yeast used in the production of beer, Saccharomyces chevalieri, Saccharomyces bayanus, and Saccharomyces italicus (Rosini et al., 1982). The wine yeasts are characterized by both an ability to ferment sugars at a high rate and a high tolerance to alcohol. S. uvarum is a well-characterized species based on morphological and biochemical characteristics.

Morphologically, S. uvarum has been described as spheroidal, ovoid, ellipsoidal, cylindrical or elongate in shape. There are also some filamentous forms. The nonfilamentous forms can occur singularly, in pairs, or clusters. Strains of this species have been determined by the size of the individual cells.

Beyond the use of morphological criteria for taxonomy, scientists have historically applied the use of fermentation of selective sugars as criteria for speciation. However, the

relative facility with which Saccharomyces spp. changes its fermentation patterns limits the utility of sugar metabolism as a criterion for speciation.

DNA homology studies have been employed as tools to delineate species within the genus but with conflicting results. Rosini et al. (1982) tested over 1,000 strains of yeast (classified as S. cerevisiae, S. uvarum, S. italicus, S. bayanus, and S. chevalieri) for DNA homology employing both reassociation and DNA composition. They found a high degree of relatedness between S. uvarum and S. bayanus. However, the DNA homology criteria was at odds with the conventional systematic criteria. The authors noted that despite the high degree of homology between S. uvarum and S. bayanus, the fermentation potential of the two strains varied substantially.

Despite the discrepancy between the phenotypic traits and genotype, there appears to be a weight of data that supports the inclusion of S. bayanus with S. uvarum. Martini and Kurtzman (1985) have presented what appears to be the definitive classification on Saccharomyces spp. They have noted the shortcomings of the current classification system and characters employed and recommended the use of DNA homology studies to develop the new scheme. The authors have proposed a reclassification of S. uvarum into S. bayanus. However, none of these organisms or other closely related species has been associated with pathogenicity toward humans or has been shown to have adverse effects on the environment.

B. Related Species of Concern

None of the above strains or other closely related species has been associated with pathogenicity toward humans or has been shown to have adverse effects on the environment.

III. HAZARD ASSESSMENT

A. Human Health Hazards

1. Colonization and Pathogenicity

S. uvarum has been used for a variety of purposes throughout history, predominantly for the processing of food. This history of use is noted by Stewart and Russell (1985) who remarked "No other group of microorganisms has been more intimately associated with the progress and well-being of the human race than Saccharomyces cerevisiae and its closely related species". Saccharomyces uvarum has been used extensively in the production

of beer and other foodstuffs, and for the production of ethanol. This legacy provides a history of long use. Within this record, there are no reported incidences of adverse effects to humans from S. uvarum. This experience is consistent not only with S. uvarum, but also with those species that are proposed to comprise the new genus S. bayanus.

The history of use of S. uvarum extends through several scenarios of exposure. Industrial application, particularly in the earlier periods of use, involved substantial human exposure both in the production facility and the research environment. Presently, the consumption of yeast (generic application) is a common source of vitamins. While the condition of "data linking the organism to disease" can be taken as an exculpatory evidence for pathogenicity, a history of significant exposure with incidence of disease in a nondebilitated condition contributes to a history of safe use.

2. Pathogenicity of closely related species for Humans

Closely related species also have a history of extensive use without significant incidence of disease. The most intensely studied of those species closely related to S. uvarum (bayanus) is S. cerevisiae. S. cerevisiae is not considered a pathogenic microorganism, but has rarely been reported as a cause of opportunistic infections. Eng et al. (1984) described five cases of such infections and reviewed the literature on eight other S. cerevisiae infections (also briefly reviewed by Walsh and Pizzo, 1988). All of the patients had underlying disease. Some of them had also received antibiotic therapy, thereby suppressing normal bacterial flora and allowing mycotic organisms to become established.

A low concern for the pathogenicity of S. cerevisiae is also illustrated by a series of surveys conducted at hospitals over the last several years. S. cerevisiae accounted for less than 1% of all yeast infections isolated at a cancer hospital and in most of the cases the organism was isolated from the respiratory system (Kiehn et al., 1980). At Yale-New Haven Hospital over the past five years, there have been 50 isolates of S. cerevisiae recovered from patients; however, most of the isolates were considered contaminants (Dynamac, 1991).

3. Toxin Production

There were no reports found in the literature that indicate that S. uvarum produces toxins to humans or animals.

4. Measure of the Degree of Virulence

Although information is not available on the potential virulence of S. uvarum, there is information available on the closely related S. cerevisiae. A number of individual virulence factors have been identified as being associated with the ability of yeasts to cause disease. The principal virulence factors associated with yeasts appear to be phospholipase A and lysophospholipase. It is believed that these enzymes enhance the ability of the yeast to adhere to the cell-wall surface and result in colonization as a first step in the infectious process. Nonpathogenic yeast had considerably lower phospholipase activities. Of a wide range of fungi assayed for phospholipase production, S. cerevisiae was found to have the lowest level of activity (Barrett-Bee et al., 1985). Therefore, based on the phospholipase virulence factor S. cerevisiae is considered a nonpathogenic yeast.

A second factor associated with virulence in yeast is the ability of a fungus to impair the host's immune capabilities. The cell walls of most fungi have the capacity to impede the immune response of the host. In a study that determined the overall pathogenicity of a number of yeasts used in industrial processes animals exposed to both high levels of S. cerevisiae, and cortisone demonstrated a greater ability of the fungus to colonize compared with those animals treated with only the yeast. However, the animals suffered no ill-effects from the introduction of S. cerevisiae, (Holzschu et al., 1979). Therefore, this study suggests that even with the addition of high levels of an immunosuppressive agent, S. cerevisiae appears to be nonpathogenic.

5. Conclusions

There is no information in the literature associating S. uvarum with pathogenicity in humans. Although the absence of data on the potential pathogenicity of an organism usually cannot serve as confirmation of lack of toxicity, the legacy of use and continuous exposure, both through ambient exposure and industrial contact, contribute to an extensive history of safe use. There is much more information available on the related species S. cerevisiae due to its more widespread use. Even with this organism, reported incidences of infection are extremely rare and invariably in individuals with existing debilitating conditions. The body of evidence clearly indicates that the S. uvarum component of S. bayanus would not be expected to produce disease states with industrial application as long as no traits that would enhance the infectivity, ability to colonize, and

virulence or toxin producing capability of organism are introduced.

S. uvarum has been used extensively for a great many years for making beer and wine and for alcohol production under conditions which at times must have resulted in considerable human exposure with no recorded instances of colonization or infection. Nor has it been reported to cause disease in animals.

Since S. uvarum has no reported history of pathogenicity during its long and extensive use, and its closely related species have but very low reported instances of infection, full exemption status is recommended.

B. Environmental Hazards

S. uvarum has been isolated from such natural sites as honey, phyllosphere, on the surfaces and inside rotten fruit, and in fruit juice. Despite the ubiquity of S. uvarum in nature there are no reports in the literature indicating that the organism is pathogenic to animals, plants or other microorganisms. Furthermore, there are no reports of this organism producing toxins against animals or plants.

IV. EXPOSURE ASSESSMENT

A. Worker Exposure

S. uvarum is considered a Class 1 Containment Agent under the National Institute of Health (NIH) Guidelines for Recombinant DNA Molecules (U.S. Department of Health and Human Services, 1986).

No data were available for assessing the release and survival specifically for fermentation facilities using S. uvarum. Therefore, the potential worker exposures and routine releases to the environment from large-scale, conventional fermentation processes were estimated on information available from eight premanufacture notices submitted to EPA under TSCA Section 5 and from published information collected from non-engineered microorganisms (Reilly, 1991). These values are based on reasonable worst-case scenarios and typical ranges or values are given for comparison.

During fermentation processes, worker exposure is possible during laboratory pipetting, inoculation, sampling, harvesting, extraction, processing and decontamination procedures. A typical site employs less than 10 workers/shift and operates 24 hours/day throughout the year. NIOSH has conducted walk-through surveys of

several fermentation facilities in the enzyme industry and monitored for microbial air contamination. These particular facilities were not using recombinant microorganisms, but the processes were considered typical of fermentation process technology. Area samples were taken in locations where the potential for worker exposure was considered to be potentially greatest, ie. near the fermentor, the seed fermentor, sampling ports, and separation processes (either filter press or rotary drum filter). The workers with the highest potential average exposures at the three facilities visited were those involved in air sampling. Area samples near the sampling port revealed average airborne concentrations ranging from 350 to 648 cfu/m³. Typically, the Chemical Engineering Branch would not use area monitoring data to estimate occupational exposure levels since the correlation between area concentrations and worker exposure is highly uncertain. Personal sampling data are not available at the present time. Thus, area sampling data have been the only means of assessing exposures for previous PMN biotechnology submissions. Assuming that 20 samples per day are drawn and that each sample takes up to 5 minutes to collect, the duration of exposure for a single worker will be about 1.5 hours/day. Assuming that the concentration of microorganisms in the worker's breathing zone is equivalent to the levels found in the area sampling, the worst-case daily inhalation exposure is estimated to range up to 650 to 1200 cfu/day. The uncertainty associated with this estimated exposure value is not known (Reilly, 1991).

B. Environmental and General Exposure

1. Fate of the Organism

S. uvarum is a normal inhabitant of soils and the surfaces of plants. S. uvarum is capable of utilizing a diverse group of substrates as carbon and nitrogen sources. The nutritional characteristics, along with the ability to produce ascospores, enhances the ability of the organism to survive in nature. to take up a wide variety of sugars and amino acids (Versar, 1992).

2. Releases

Estimates of the number of S. uvarum organisms released per production batch are tabulated in Table 1. The minimally controlled scenario assumes no treatment of the fermentor off-gas and assumes 100-fold (2 log) reduction of the maximum cell density of the fermentation broth resulting from inactivation (Reilly, 1991). The containment criteria required for the full exemption scenario assume the use of in-line filters to treat vent gases and a 99% removal efficiency under normal operating conditions. They also assume an overall 6-log reduction relative

to the maximum cell density of the fermentation broth resulting from inactivation steps (Reilly, 1991).

TABLE 1. Estimated Number of Viable *Saccharomyces uvarum* Organisms Per Production Batch

| Release Media | Minimally Controlled (cfu/day) | Full Exemption (cfu/day) | Release (days/year) |
|--------------------|--------------------------------------|---------------------------------|------------------------|
| Air Vents | $2 \times 10^8 - 1 \times 10^{11}$ | $2 \times 10^6 - 1 \times 10^9$ | 350 |
| Rotary Drum Filter | 250 | 250 | 350 |
| Surface Water | 7×10^{12} | 7×10^8 | 90 |
| Soil/Landfill | 7×10^{14} | 7×10^{10} | 90 |

Source: Reilly, 1991

3. Air

While there is no specific information on the survival of *S. uvarum* in the atmosphere, the organism's ability to form ascospores suggests that survival rates would be very good. Environmental exposure would occur as the organisms drift to earth and take up residence in the soil. Human exposure is expected to be low, since the numbers of organisms released would be quickly diluted in the atmosphere (Versar, 1992).

4. Water

S. uvarum released to water would be expected to survive publicly owned treatment works (POTW) treatment and discharge. Surface water concentrations of organisms were estimated using the 10% and 50% flow values for SIC Code 2082 to 2085 (Malt beverages, wines, brandy, brandy spirits, distilled and blended liquors) that release to surface water. The SIC code flow was estimated using 128 indirect (facilities that send their waste to a POTW) and direct (facilities that have an NPDES permit to discharge to surface water) dischargers. Discharger data were extracted from the IFD (Industrial Facilities Dischargers) database and surface water flow data were taken from the RXGAGE database, maintained by the EPA. These data, which were partitioned into percentile rankings and flows for the 10th percentile (small river) and 50th (average river), were extracted and used for the exposure calculations. Flow is expressed in Millions of Liters/Day (MLD). Mean Flow is the average flow value, and 7Q10 flow is the lowest flow observed over 7 consecutive days during a 10 year period. Concentrations of

microorganisms in surface water are calculated for both the minimally controlled and the full exemption scenarios (LaVeck, 1991).

TABLE 2. *Saccharomyces uvarum* Concentrations in Surface Water

| Flow | Receiving Stream Flow (MLD*) | | Organisms (cfu/l) | |
|----------------------|------------------------------|------|----------------------|-------------------|
| | Mean | Q710 | Mean | Q710 |
| Minimally Controlled | | | | |
| 10th Percentile | 141 | 5.11 | 5.0×10^4 | 1.4×10^6 |
| 50th Percentile | 2535 | 186 | 2.8×10^3 | 3.8×10^4 |
| Full Exemption | | | | |
| 10th Percentile | 141 | 5.11 | 5.0×10^0 | 1.4×10^2 |
| 50th Percentile | 2535 | 186 | 2.8×10^{-1} | 3.8×10^0 |

*MLD = million liters per day

Source: Versar, 1992

5. Soil

Since soil is a natural habitat for *S. uvarum*, it would be expected to survive well in soil. These releases could result in human and environmental exposure (Versar, 1992). However, it is anticipated that industrial strains which have adapted to well-defined media would be less competitive than the original wild-type strain (Sayre, 1992).

V. INTEGRATED RISK ASSESSMENT

A. Discussion

There is an extensive history of use of and exposure to *S. uvarum* with no record of adverse effects to the environment or human health. Yeast has been used for centuries as a leavening for bread and fermenter of beer without records of virulence.

S. uvarum is not a plant or animal pathogen. Although it has been used extensively in the fermentation and food processing industry, *S. uvarum* has not been found to be associated with disease conditions in plants or animals.

There is the likelihood that the taxonomy of S. uvarum will be modified in the future. The problem with taxonomy is not considered a risk issue. There are no closely related yeasts which pose a human or ecological hazard. Worker and non-occupational human exposures are not expected to be substantial based on containment requirements for Biosafety Level 1 required by NIH guidelines. The requirements for containment for exemption are expected to further diminish exposure.

B. Recommendation

Despite the lack of taxonomic clarity of S. uvarum, concerns for ecological and human health risks are low. Therefore, it is recommended that this organism be granted exemption for closed systems applications in which requisite criteria are applied.

VI. REFERENCES

21 CFR 184.1983.

Anderson, A. 1992. Yeast genome project: 300,000 and counting. *Science* 256:462.

Barrett-Bree, Y. Hayes, R.G. Wilson, and J.F. Ryley. 1985. A comparison of phospholipase activity, cellular adherence and pathogenicity of yeasts. *J. Gen. Microbiol.* 131:1217-1221.

Bigelis, R. 1985. Primary metabolism and industrial fermentations. in *Gene Manipulations in Fungi*. J.W. Bennet and L.L. Lasure (ed.) Academic Press, New York, NY. PP 357-

Brondz, I., and I. Olsen. 1990. Multivariate analyses of cellular carbohydrates and fatty acids of *Candida albicans*, *Torulopsis glabrata*, and *Saccharomyces cerevisiae*. *J. Clin. Microbiol.* 28:1854-1857.

Buesching, W.J., K. Kurek, and G.D. Roberts. 1979. Evaluation of the modified API 20C system for identification of clinically important yeasts. *J. Clin. Microbiol.* 9565-569.

Bussey, H., T. Vernet, and A.-M. Sdicu. 1988. Mutual antagonism among killer yeasts: competition between K1 and K2 killers and a novel cDNA-based K1-K2 killer strain of *Saccharomyces cerevisiae*. *Can. J. Microbiol.* 34:38-44.

Dynamac. 1990. Evaluation of microorganisms for possible exemption under TSCA Section 5. Unpublished, U.S. Environmental Protection Agency, Washington, D.C.

Dynamac. 1991. Human health assessment for *Saccharomyces cerevisiae*. Unpublished, U.S. Environmental Protection Agency, Washington, D.C.

Holzschu, D.L., F.W. Chandler, L. Ajello, and D.G. Ahearn. 1979. Evaluation of industrial yeasts for pathogenicity. *Sabouraudia* 17:71-78.

Kiehn, T.E., F.F. Edwards, and D. Armstrong. 1980. The prevalence of yeasts in gastrointestinal inoculation in antibiotic treated mice. *Sabouraudia* 21:27-33.

Kough, J. 1990. Clarification of taxonomic questions on *Saccharomyces cerevisiae*. Unpublished, U.S. Environmental Protection Agency, Washington, D.C.

Martini, A. and C. Kurtzman. 1985. Deoxyribonucleic acid relatedness among species of the genus *Saccharomyces sensu stricto*. *Int. J. System. Bacteriol.* 35:508-511.

Nobre, G.N., and A.F. Ferreira. 1986. Enhancement of *Streptococcus faecalis* infection and complement depletion in yeast-treated mice. *J. Gen. Microbiol.* 132:1277-1281.

Organization for Economic Cooperation and Development. 1986. *Recombinant DNA Safety Considerations*. Paris, France.

Phaff, H., M. Miller, and E. Mrak. 1966. *The life of yeasts*. Harvard University Press. Boston, MA.

Reilly, B. 1991. Analysis of environmental releases and occupational exposure in support of the proposed TSCA 5(h)(4) exemption. Unpublished, U.S. Environmental Protection Agency, Washington, D.C.

Rosini, G., F. Federici, A.E. Vaughn, and A. Martini. 1982. Systematics of the species of the yeast genus *Saccharomyces* associated with the fermentation industry. *Eur. J. Appl. Biotechnol.* 15:188-193.

Sayre, P. 1991. Environmental hazard assessment of *Saccharomyces cerevisiae* for proposed 5(h)4 exemption. Unpublished, U.S. Environmental Protection Agency, Washington, D.C.

Stewart, G.C. and I. Russell. 1985. The biology of *Saccharomyces*, pp. 511-536. In A.L. Demain and N.A. Solomon, (eds.), *Biology of industrial organisms*. Benjamin Cummins Publishers, Menlo Park.

Tao, J., I. Ginsberg, N. Banerjee, W. Held, Y. Koltin, and J.A. Bruenn. 1990. *Ustilago maydis* KP6 Killer toxin: structure, expression in *Saccharomyces cerevisiae*, and relationship to other cellular toxins. *Mol. Cell. Biol.* 10:1373-1381.

Thayer, D.W. 1990. Personnel communication with C. Felkner. (Cited in *Dynamac*, 1990).

U.S. Department of Health and Human Services. 1986. Guidelines for research involving recombinant DNA molecules, Appendix F. 51 FR 16971.

Van der Walt, J. 1971. *Saccharomyces*, pp. 597-605. In J. Lodder, (ed.), *The yeasts, a taxonomic study*. North Holland Publ. Co. Amsterdam.

Versar. 1992. Screening level exposure assessment of *Saccharomyces uvarum* for 5(h)(4) exemption under the proposed biotech rule. Unpublished, U.S. Environmental Protection Agency, Washington, D.C.

Wolochow, H., G.J. Hildebrand, and C. Lamanna. 1961. Translocation of microorganisms across the intestinal wall of the rat: effect of microbial size and concentration. *J. Infect. Dis.* 116:523-528.